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COMPARISON OF MINITEK AND CONVENTIONAL METHODS FOR THE BIOCHEMICAL CHARACTERIZATION OF ORAL STREPTOCOCCI

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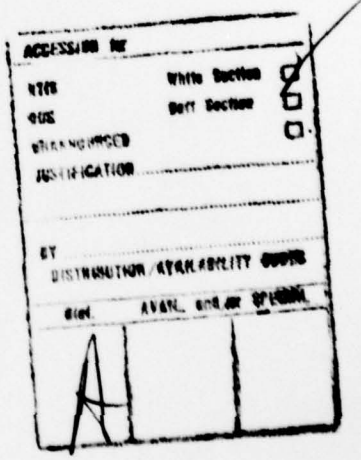

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ABSTRACT

Fifty-six strains of viridans streptococci were tested in the Minitek system (BBL, Cockeysville, Md.) and the results were compared with those obtained by conventional methods. An overall agreement of 98.9% was obtained when the Minitek tests were performed as follows: (1) All disks were incubated anaerobically for 48 h except for esculin which required 5 - 7 days. (2) The arginine disks were overlaid with 0.1 ml sterile mineral oil even though incubated anaerobically. (3) The Voges-Proskauer tests were performed under aerobic and anaerobic conditions. (4) All tests for carbohydrate fermentation except for raffinose and salicin were read following the addition of 2 - 3 drops of 0.025% phenol red. Of all the tests performed, only the fermentation of starch resulted in such poor agreement as to be considered unreliable.

Oral streptococci consist of a heterologous group of poorly defined organisms commonly referred to as the viridans streptococci, a term not totally descriptive since some oral streptococci may occasionally produce complete hemolysis or be nonhemolytic on blood agar containing sheep red blood cells (6). Attempts to develop comprehensive schemes for serological classification of viridans streptococci have failed, and no correlation between serological and biochemical characteristics has been observed (8, 9, 13, 14, 17). Speciation of viridans streptococci is possible on the basis of physiological characteristics as shown by Facklam (2) who reported a 97% success rate in the identification of 1227 clinical and 80 stock strains. The biochemical scheme proposed by Facklam is applicable after alpha and gamma hemolytic strains of groups B, D, N, and Q are serologically identified and excluded from the key.

Commonly used tests such as sugar fermentations, hydrolysis of esculin and arginine, and tests for the production of indole and urease in tubed media are laborious and time consuming. To overcome these difficulties, miniaturized systems have been recently developed and shown to be accurate and dependable for characterization of *Enterobacteriaceae* (4,7), lactobacilli (3), and anaerobes (5, 10, 15). Because of the reported important role of biochemical characteristics in the identification of oral streptococci, we have investigated the reliability of the commercially available identification system, Minitek, (BBL, Cockeysville, Md.) by comparing it with conventional methods.

MATERIALS AND METHODS

Bacteria. Strains of microorganisms used in this study are listed in Table 1. The majority of strains were obtained from the American Type Culture Collection, Rockville, Md. Additional strains were kindly supplied by B. Gunn, Walter Reed Army Medical Center, Washington, DC; K.C. Gross, The New York Hospital, New York, N.Y.; L.A. Thomson, National Institute of Dental Research, Bethesda, Md.; and R.R. Facklam, Center for Disease Control (CDC), Atlanta, Ga. All cultures were maintained on 5% sheep blood agar plates at 37°C in a GasPak jar containing disposable GasPak generators (H_2+CO_2) (BBL, Cockeysville, Md.) and activated catalyst replacement charges (BBL). Lyophilized cultures (ATCC, Rockville, Md.) were reconstituted with brain heart infusion broth (Difco, Detroit, Mich.).

Minitek Procedure. The Minitek system (BBL) includes paper substrate disks (a choice of 35 is available) which are placed into wells stamped in plastic plates. Using this system the tests were performed as follows: Bacterial growth (24 h) from the surface of a blood agar plate was removed with a dry sterile cotton swab and suspended in 1 - 1.2 ml of Minitek enteric and nonfermenter broth. The resulting suspensions were dispensed with a 0.05 ml repeating dispenser gun into the individual wells containing the appropriate test disks, overlaid with two drops of sterile mineral oil (API, Plainview, N.Y.) in accordance with the manufacturer's instructions, and incubated aerobically at 37°C in the Minitek humidor for 48 h. A similar procedure was followed using the Minitek anaerobe broth, which is a supplemented trypticase peptone medium based on the Lombard Dowell formulation (10). Tests performed using this medium were carried out under anaerobic conditions (37°C for 48 h in a GasPak jar)

without an oil overlay. All tests were performed in duplicate.

After incubation, the results were examined before and after addition of 2 - 3 drops of 0.025% aqueous phenol red. A color change to yellow or light orange was interpreted as a positive reaction for aerobically incubated streptococci. Color change to yellow with no tint of orange indicated a positive reaction for anaerobically incubated streptococci.

Conventional Method. Acid production from carbohydrates was tested in tubes containing 5 ml of phenol red broth base (Difco Laboratories, Detroit, Mich.) pH 7.2. The media were autoclaved for 15 min at 121°C before being supplemented with filter sterilized sugars (Difco) and soluble starch (Difco) solutions to give a 1% final concentration. Organisms were grown in tryptic soy broth (Difco) for 24 h at 37°C, and incubated aerobically except for strains of *Streptococcus anginosus-constellatus* which were incubated under 5% CO_2.

Results were recorded at 48 h, 7, and 14 days. Uninoculated media incubated 14 days served as controls for color comparison. A color change of the media from red to yellow was recorded as a positive reaction. The medium of Niven, Smiley, and Sherman (11) was used for the determination of ammonia production from L-arginine. Development of an orange-yellow color following addition of 0.1 ml of Nessler's reagent to the 48 h culture indicated ammonia production. Christensen's urea broth, Simmons' citrate agar, and tryptic nitrate medium (Difco), prepared according to the manufacturer's recommendations, were inoculated and the results examined as described by Vera and Dumoff (16) after incubation at 37°C

for 48 h. Esculin broth was prepared as described previously (15). A black precipitate after 48 h of incubation indicated a positive reaction. Difco MR-VP broth was inoculated for the Voges-Proskauer test which was performed by the O'Meara modified method (12). Since inulin disks are not presently manufactured by BBL for use in the Minitek system, results from conventionally prepared inulin broth were utilized in Facklam's key for the identification of viridans streptococci.

RESULTS

Although mannitol, lactose, inulin, arginine, esculin, raffinose, sorbitol, and acetoin (Voges-Proskauer) have been described as differentiating tests for oral streptococci (2,3), seventeen additional tests (Table 2) were performed and the results compared with conventional methods to more fully assess the applicability of the Minitek system. The percent of tests in agreement with conventional reactions are listed in Table 2.

By comparing two methods of performing the Minitek system with conventional methods commonly used for characterizing streptococcus species (Table 2), we were able to demonstrate an overall agreement of 98.9% when the Minitek tests were performed as follows: (1) All disks were incubated anaerobically for 48 h except for esculin which required 5 - 7 days. (2) The arginine disks were overlaid with 0.1 ml sterile mineral oil even though incubated anaerobically. (3) The Voges-Proskauer tests were performed under aerobic and anaerobic conditions. (4) All tests for carbohydrate fermentation except for raffinose and salicin were read following the addition of 0.025% phenol red.

Of all the tests performed, only the fermentation of starch resulted in such poor agreement as to be considered unreliable. Of all other tests performed under optimal conditions, the poorest agreement (87.5%) occurred with cellobiose.

Following anaerobic incubation, the addition of 2-3 drops of 0.025% aqueous phenol red (pH 7.2) to the carbohydrate disks was found to greatly facilitate interpretation by eliminating borderline colors and thereby improving the overall agreement with conventional methods by 1.3%. Only the raffinose and salicin tests gave better agreement when no phenol red was added. Results (Table 2) show that the Minitek-anaerobic method read with phenol red exhibited an overall agreement of 98.2% with the conventionally tubed media, whereas, the aerobic Minitek-oil method read with phenol red exhibited an agreement of 94.9%. Following the use of phenol red, a 5.1% decrease in overall agreement between conventional and aerobic-oil Minitek results was observed; but only a 1.8% decrease between conventional and anaerobic-Minitek was noted. This represents a 3.3% increase in agreement with conventional methods for the Minitek-anaerobic method.

A 100% agreement was observed for the urea test which was included in this study since 50% of the strains of *Streptococcus salivarius* have been reported (6) to be urea positive.

DISCUSSION

We have observed frequent false negative reactions associated with certain species of oral streptococci (particularly strains of *S. anginosus-constellatus*) in the Minitek system when performed under aerobic conditions

even though the disks were overlaid with oil. Anaerobic incubation for 48 h eliminated these false negative reactions except for the esculin test which required an extended incubation of 5 - 7 days. An accurate esculin test was essential in Facklam's key for the differentiation of *S. anginosus-constellatus* from *Streptococcus morbillorium*. The more luxuriant growth of most streptococci occurring under anaerobic conditions decreased the incubation period required for completion of the tests. Accurate test results were observed for *Streptococcus mutans* (ATCC 25175) within 5 h under anaerobic conditions in Lombard Dowell media, whereas, 48 h were required when inoculated Minitek enteric and nonfermenter broth was incubated aerobically. Under anaerobic conditions, the arginine disk required a 0.1 ml mineral oil overlay to prevent escape of ammonia from the media which resulted in false negative or borderline reactions. Under these conditions a 100% agreement with the conventional arginine test was obtained.

Since the Voges-Proskauer test depended upon the oxidation of acetylmethylcarbinol in alkaline media to form diacetyl (1), dissolved oxygen existing in the aerobically incubated media enhanced the pink color required for a positive reaction. Consequently, organisms capable of growing aerobically gave the strongest positive reaction under aerobic conditions. Due to poor growth aerobically, the microaerophilic oral streptococci required an anaerobic environment to facilitate the abundant growth necessary for a positive Voges-Proskauer reaction. Therefore, it is recommended that this test be performed both aerobically and anaerobically, with a color change under either condition considered a

positive reaction for the Voges-Proskauer test. The difficulty encountered in reading an anaerobically incubated Voges-Proskauer test may be partially overcome through aeration by vigorous stirring following the addition of reagents.

Kiehn *et al.* (7) reported a 97.2% agreement for the Minitek urea test when compared with the conventional Christensen's urea media for urease produced by the *Enterobacteriaceae*. They observed false negative urea reactions with *Serratia*, *Enterobacter*, and *Citrobacter* species. In a similar study, Hansen *et al.* (5) reported a 98.9% agreement. Of the oral streptococci, only certain strains of *S. salivarius* were urease positive (6). We found 42% of the *S. salivarius* strains tested to be urease positive, and observed a 100% agreement in results obtained by comparing the two methods.

Of all the tests performed only the fermentation of starch resulted in such poor agreement as to be considered unreliable.

Although 100% agreement for all substrate disks and all organisms may not be attainable, a 98.2% agreement is sufficiently high as to offer a reasonable substitute for the more expensive conventional methods, the utilization of which is more cumbersome and time consuming. As is reasonably expected, proper application and familiarity with the system is required. It has been suggested (2) that past failures to successfully use biochemical tests to differentiate oral streptococci occurred because sufficiently large groups of tests were not used. The Minitek system allows the small laboratory easy access to many tests since the manufacturer claims a two year shelf life for the disks which conveniently

require very small amounts of refrigerator/incubator space. The miniaturized Minitek micromethod provides a faster method for the identification of oral streptococci easily performed by the clinical microbiology laboratory.

MILITARY DISCLAIMER

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

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TABLE 1. STRAINS OF STREPTOCOCCI TESTED

IDENTIFICATION WITH FACKLAM'S KEY	IDENTIFICATION NUMBER	SENDER'S IDENTIFICATION
S. salivarius	ATCC 9222	S. salivarius
	ATCC 9758	S. salivarius
	ATCC 9759	S. salivarius
	ATCC 13419	S. salivarius
	ATCC 27945	S. salivarius
	ATCC 25975	S. salivarius
	ATCC 31067	S. salivarius
	CDC SS-262[a]	S. salivarius
	206[b]	S. salivarius
	208[b]	S. salivarius
	5[b]	S. sanguis biotype I
	4/1212a[b]	S. sanguis biotype I
S. mutans	ATCC 10035	S. sp
	ATCC 19641	S. sp
	ATCC 19642	S. sp
	ATCC 19643	S. sp
	ATCC 19645	S. sp
	ATCC 25175	S. mutans
	ATCC 27351	S. mutans
	ATCC 27352	S. mutans
	ATCC 27607	S. mutans
	ATCC 27947	S. mutans
S. sanguis biotype I	ATCC 8144	S. sp Lancefield H
	ATCC 10556	S. sanguis
	ATCC 10558	S. sanguis
	ATCC 12396	S. sp Lancefield H
	CDC SS-910[a]	S. sanguis biotype I
	6[b]	S. sanguis biotype I
	11[b]	S. sanguis biotype I
S. sanguis biotype II	ATCC 903	S. mitis
	ATCC 6249	S. mitis
	ATCC 10557	S. sanguis
	ATCC 15914	S. mitis type VII
	CDC SS-911[a]	S. sanguis biotype II
	JC-74[c]	S. sanguis biotype II
S. mitis	ATCC 4782	S. sp
	ATCC 9811	S. mitis
	ATCC 15909	S. mitis type I
	ATCC 15910	S. mitis type II
	ATCC 15911	S. mitis type III
	ATCC 15912	S. mitis type IV
	ATCC 15913	S. mitis type VI
S. anginosus-constellatus	ATCC 27823	S. constellatus
	CDC SS-1111[a]	S. anginosus-constellatus
	P-651[d]	S. milleri
	B-1823[d]	S. milleri
	E-5033[d]	S. milleri
	C-3654[d]	S. milleri
	P-1914[d]	S. milleri
	B-4534[d]	S. milleri
	409[b]	S. milleri
S. MG-intermedius	ATCC 27335	S. intermedius
	ATCC 9895	S. mitis type V
	CDC SS-899[a]	S. MG-intermedius
	408[b]	S. milleri
	404[b]	S. milleri

Strains obtained from:

[a] CDC collection.
[b] B. Gunn, Walter Reed Army Medical Center, Washington, DC
[c] L. A. Thomson, National Institute of Dental Research, Bethesda, MD
[d] K. C. Gross, Laboratory of Microbiology, The New York Hospital, New York, NY

TABLE 2. PERCENT AGREEMENT FOR RESULTS OBTAINED WHEN 56 STREPTOCOCCAL STRAINS WERE TESTED BY BOTH THE CONVENTIONAL AND MINITEK SYSTEMS.

	AEROBIC - OIL OVERLAY (except as indicated)		ANAEROBIC - NO OIL OVERLAY (except as indicated)	
	NO PHENOL RED	PHENOL RED	NO PHENOL RED	PHENOL RED
ARABINOSE	48.2	92.8	100	100
CELLOBIOSE	78.2	75.0	75.0	87.5
DEXTROSE	92.8	89.3	100	100
GLYCEROL	98.2	100	100	100
INOSITOL	98.2	100	85.7	100
LACTOSE	98.2	91.4	92.8	98.2
MALTOSE	91.4	91.4	100	100
MANNITOL	98.2	100	96.4	100
MANNOSE	100	91.0	100	100
MELIBIOSE	98.2	98.2	98.2	100
RAFFINOSE	98.2	100	96.4	92.8
RHAMNOSE	100	100	98.2	100
SALICIN	92.8	87.5	96.4	85.7
SORBITOL	96.4	98.2	91.1	96.4
STARCH	8.9	0	16.1	8.9
SUCROSE	96.4	96.4	100	100
TREHALOSE	91.4	91.4	100	100
XYLOSE	100	100	100	100
ARGININE	89.3	N/A[f]	100[b]	N/A
CITRATE	100[a]	N/A	100	N/A
ESCULIN	86.0[a]	N/A	100	N/A
INDOLE	100[a]	N/A	100	N/A
NITRATE	100[a]	N/A	100	N/A
UREA	100	N/A	100[b]	N/A
VOGES-PROSKAUER	98.8[a]	N/A	96.4	N/A
MEAN (Excluding Starch)	93.5	94.9[e]	96.9	98.2[d] 98.9[e]

[a] No oil overlay.

[b] Oil overlay.

[c] Mean calculated using results for non-carbohydrate substrates (Arginine - Voges-Proskauer) from the aerobic - oil - no phenol red column.

[d] Mean calculated using results for non-carbohydrate substrates (Arginine - Voges-Proskauer) from the anaerobic - no oil - no phenol red column.

[e] Mean value becomes 98.9% when tests for raffinose and salicin are read in the absence of phenol red.

[f] Not Applicable.

LITERATURE CITED

1. Barrett, M.M. 1936. The intensification of the Voges-Proskauer reaction by the addition of α-naphthol. J. Pathol. 42:441-454.
2. Facklam, R.R. 1977. Physiological differentiation of viridans streptococci. J. Clin. Microbiol. 5:184-201.
3. Gilliland, S.E. and M.L. Speck. 1977. Use of the Minitek system for characterizing lactobacilli. Appl. and Environ. Microbiol. 33:1289-1292.
4. Hansen, S.L., D.R. Hardesty, and B.M. Meyers. 1974. Evaluation of the BBL Minitek system for the identification of *Enterobacteriaceae*. Appl. Microbiol. 28:798-801.
5. Hansen, S.L. and B.J. Stewart. 1976. Comparison of API and Minitek to Center for Disease Control methods for the biochemical characterization of anaerobes. J. Clin. Microbiol. 4:227-231.
6. Hardie, J.M. and G.H. Bowden. 1976. Physiological classification of oral viridans streptococci. J. Dent. Res. 55 (Spec.Issue A):A166-A176.
7. Kiehn, T.E., K. Brennan, and P.D. Ellner. 1974. Evaluation of the Minitek system for identification of *Enterobacteriaceae*. Appl. Microbiol. 28:668-671.
8. Lancefield, R.C. 1925. The immunological relationship of streptococcus viridans and certain of its chemical fractions. I. Serological reactions obtained with antibacterial sera. J. Exp. Med. XLII:337-395.
9. Lancefield, R.C. 1925. The immunological relationship of streptococcus viridans and certain of its chemical fractions. II. Serological reactions obtained with antinucleoprotein sera J. Exp. Med. XLII:397-412.

10. Lombard, G.L., A.Y. Armfield, F.S. Thompson, M.D. Stargel, and V.R. Dowell, Jr. 1975. Improved procedure for biochemical characterization of anaerobic bacteria. Abstr. Annu. Meet. Am. Soc. Microbiol. C 38, p.33.

11. Niven, C.F., Jr., K.L. Smiley, and J.M. Sherman. 1942. The hydrolysis of arginine by streptococci. J. Bact. 43:651-660.

12. Paik, George. 1970. Reagents, stains, and test procedures, p. 681. *In* J.E. Blair, E.H. Lennette and J.P. Truant (ed.), Manual of Clinical Microbiology. Williams and Wilkins Co., Baltimore.

13. Selbie, R.R., E.D. Simon, and R.G.M. Robinson. 1949. Serological classification of viridans streptococci from subacute bacterial endocarditis, teeth, and throats. Br. Med. J. ii:667-672.

14. Soloway, M. 1942. A serological classification of viridans streptococci with special reference to those isolated from subacute bacterial endocarditis. J. Exp. Med. 76:109-126.

15. Stargel, M.D., F.S. Thompson, S.E. Phillips, G.L. Lombard, and V.R. Dowell, Jr. 1966. Modification of the Minitek miniaturized differentiation system for characterization of anaerobic bacteria. J. Clin. Microbiol. 3:291-301.

16. Vera, H.C. and M. Dumoff. 1970. Culture media, p. 650. *In* J.E. Blair, E.H. Lennette, and J.P. Truant (ed.), Manual of Clinical Microbiology, 1st ed. American Society for Microbiology, Bethesda, Md.

17. Williamson, C.K. 1964. Serological classification of viridans streptococci from the respiratory tract of man, p. 607-622. *In* C.A. Leone (ed.), Taxonomic Biochemistry and Serology. Ronald Press, N. Y.